Absence of Goniodysgenesis in Patients with Chromosome 13Q Microdeletion-Related Microcoria

Gerth-Kahlert, Christina ; Maggi, Jordi ; Töteberg-Harms, Marc ; Tiwari, Amit ; Budde, Birgit ; Nürnberg, Peter ; Koller, Samuel ; Berger, Wolfgang

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Report

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Absence of Goniodysgenesis in Patients with Chromosome 13Q Microdeletion-Related Microcoria

Congenital microcoria (MCOR; MIM156600) is a rare developmental ocular disorder of the anterior segment (AS) that is caused by submicroscopic deletions on the long arm of human chromosome 13 (13q32.1).[1] All deletions reported thus far encompass 2 genes, *TGDS* and *GPR180*.[1,2] The ocular phenotype was not reproducible in the gpr180$^{-/-}$ or gpr180$^{+/-}$ mouse model.[1] Iridocorneal angle dysgenesis usually is described in patients with MCOR.[1,3,4] Histopathologic studies have revealed thick juxtacanalicular connective tissue consisting of trabecular cell layers embedded in the extracellular matrix of fibrous and amorphous substances similar to patients with goniodysgenetic glaucoma.[3] Onset of MCOR-associated glaucoma ranges from childhood, as early as 6 years of age,[2] to late-onset in the third to fourth decade of life, caused by goniodysgenesis as shown by histopathologic analysis.[3] Herein we present 3 patients with a large and unreported 13q32.1 deletion associated with MCOR without goniodysgenesis and glaucoma to demonstrate the phenotype variability.

This was a retrospective single-center chart review of 3 affected patients of a nonconsanguineous family. All patients gave written consent to analyze their data. The research adhered to the tenets of the Declaration of Helsinki. The Cantonal Ethics Committee of Zurich confirmed the exempt status of this study. All patients underwent a detailed eye examination. Anterior segment spectral-domain OCT (Spectralis; Heidelberg Engineering GmbH, Heidelberg, Germany) was performed in the index patient and her brother.

Genetic analyses of the 3 patients and the unaffected father of the index patient were performed stepwise as follows from Clinical Laboratory Improvement Amendment (CLIA)-approved laboratories: (1) whole-exome sequencing, (2) copy number analysis using the CytoScan HD Array from Affymetrix (ATLAS Biolabs GmbH, Berlin, Germany), (3) long-range polymerase chain reaction (PCR) amplification of the deletion breakpoint fragments, (4) deletion breakpoint mapping by conventional PCR, and (5) Sanger sequencing of the junction fragments.

The index patient, her brother, and her mother were examined at the ages of 27 years, 36 years, and 59 years, respectively. All 3 patients reported having small pupils since childhood. Myopic corrections were prescribed to all patients from early childhood onward. No other ocular or extraocular malformations or systemic diseases were diagnosed. Family history revealed a similar condition in the maternal grandfather of the index patient and in 2 of his siblings, although no consanguinity was known.

Ocular Morphologic Features and Function

All patients demonstrated reduced visual acuity ranging from 0.6 to 0.16 (decimal Snellen). Myopia-associated retinal changes with choroidal neovascularization treated with photodynamic therapy in the right eye and atrophic macular changes in the left eye led to severe vision loss in patient III:2 in addition to possible pre-existing amblyopia. No corneal or lenticular abnormalities were visible. Phacoemulsification cataract extraction with intraocular lens implantation was performed in patient IV:2 in the right eye at 36 years of age and in his mother (III:2) in both eyes at 51 years of age. Iris appearance was characteristically abnormal in all patients: pupil diameter of less than 2 mm (except in the eyes after cataract surgery), minimal iris pigmentation, stromal thinning with corresponding transillumination, featureless iris with poorly developed collarettes and crypts, deep iris root, and increased visibility of the iris vascularization. The iridocorneal angle was minimally pigmented and open (Shaffer 3° to 4°). The iris root was positioned just below Schwalbe's line. Hence, the ciliary body band was visible in its superior part (Fig 1). Pupils responded minimally to topically administrated tropicamide eye drops. Intraocular pressure was less than 15 mmHg in all patients. Anterior segment OCT confirmed the very thin iris stroma beginning in the mid periphery and extending toward the angle with continuously preserved posterior pigmented epithelium without signs of goniodysgenesis. High-resolution spectral domain AS OCT and swept-source AS OCT provide valuable information of the AS, especially the anterior chamber angle. It is even possible to visualize Schlemm's canal. Hence, it provides information on the angle width, location of iris insertion in comparison to Schlemm's canal and scleral spur, and angle anatomy, for example, angle recession. However, the depth of penetration of OCT is limited. Therefore, it usually is not possible to visualize structures behind the iris, for example, iris cysts. For these entities, ultrasound biomicroscopy would be the diagnostic tool of choice (Fig 1). The optic nerve showed a tilted configuration in all 3 patients with a small cup-to-disc ratio of 0.2 in patients IV:1 and IV:2, but was not measurable because of tilting in patient III:2. The size of the optic nerve measured by the SD OCT measurement tool varied from small (1.5 mm in diameter in patient IV:2), to horizontal-oval shape (VI:1: right eye, 2.16×1.77 mm; left eye, 2.06×1.69 mm, horizontal × vertical, respectively), to upright-oval shape (III:2: right eye, 1.28×1.98 mm; left eye, 1.5×2.18 mm, horizontal × vertical, respectively; Fig S1, available at www.ophthalmologyglaucoma.org). Visual field results are available in 1 patient (III:2) that show central to paracentral scotoma corresponding to maculopathy and a temporal scotoma corresponding to the myopic fundus changes in the left eye more than in the right eye (Fig S1).

Genetic Analyses

On whole-exome sequencing data analysis, no pathogenic variant was found in literature-selected genes, which have been associated with different forms of microcoria. Copy number analysis using Copy Number estimation by a Mixture Of PoissonS (cn.MOPS)[5] suggested a heterozygous deletion of the neighboring genes *TGDS* and *GPR180*. Consequently, a copy number analysis on CytoScan HD data using ChAS version 3.1 was performed for the index patient and the affected mother, which confirmed the presence of an 80- to 84-kb deletion on the long arm of chromosome 13 (13q32.1) including both genes. Primers were designed to amplify the breakpoint regions by multiplex PCR. The PCR products were run on a Bioanalyzer DNA 7500, which revealed a third product of

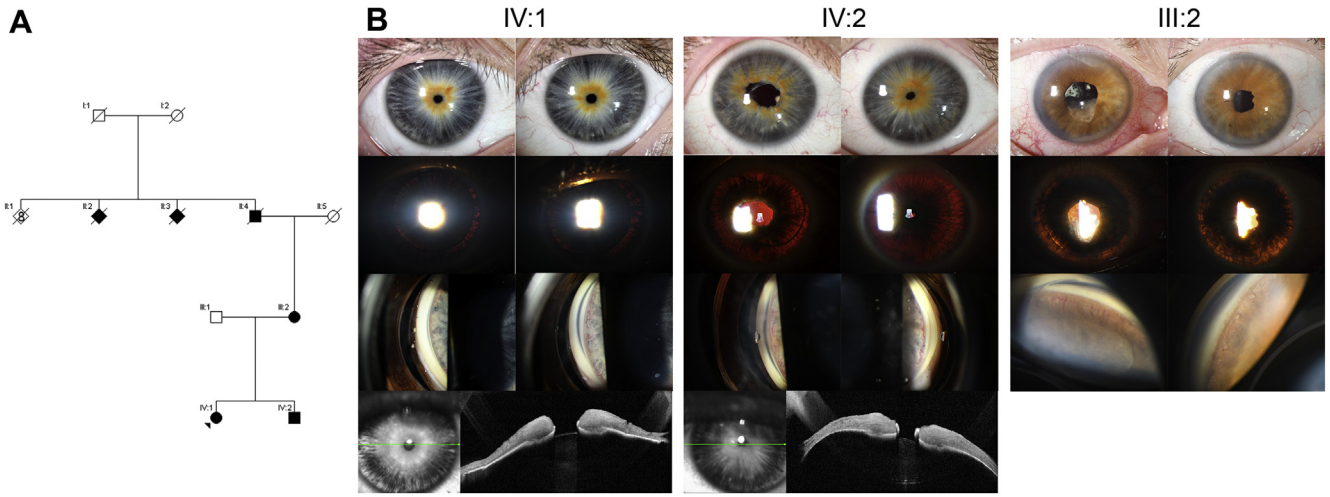

Figure 1. A, Pedigree of 3 affected patients. **B**, Anterior segment phenotype in iris photography of the 3 affected patients demonstrating reduced iris pigmentation and iris stroma thinning, with visible transillumination on indirect illumination. The iridocorneal angle in all 3 patients is open, low pigmented, and without signs of goniodysgenesis. Horizontal anterior spectral-domain OCT scans through the pupillary level of patients IV:1 and IV:2 (left eye shown only) revealing a preserved posterior iris pigment, but thinned iris stroma.

approximately 4.2 kb in length in all affected patients that was not present in the unaffected father or in an unrelated control. The 4.2-kb fragment was sequenced on a MiSeq using the Nextera XT library kit (Illumina). A STAR aligner[6] was used to split the junction reads and visualize the exact breakpoints. Sanger sequencing confirmed the 82 656-bp deletion (Chr13(GRCh37):g.95209609_95292265del; Fig S2, available at www.ophthalmologyglaucoma.org). The deletion was not present in 278 in-house ethnically matched controls without any apparent eye condition.

A temporal sequence of differentiation events is characteristic for human iris development. The dilator pupillae muscle development occurs at approximately 6 months of gestation, which is later than the sphincter pupillae muscle growth. The dilator pupillae muscle extends radially to the pupil from the anterior or pigmented epithelial layer of the iris. Interruption of the anterior and stromal iris development will be associated with dilator muscle hypotrophy and small pupils as present in patients with MCOR. Variable phenotype severity may be associated with the reported deletion. All deletions described to date include the 2 genes *TGDS* and *GPR180*, and it is not yet clear whether a deletion of both genes is associated with the MCOR phenotype or if the deletion or mutation of one of them would be sufficient to elicit the phenotype. Interestingly, a 2-generation family with unique heterozygous *GPR180* nonsense mutations associated with iridocorneal angle dysgenesis but normal pupillary response and absence of iris transillumination was described.[1] The cases presented herein add to the phenotypic spectrum associated with *TGDS* or *GPR180* gene deletions, ranging from isolated goniodysgenesis, to microcoria with iris hypoplasia but without goniodysgenesis,[7] to the full MCOR phenotype with glaucoma. A goniodysgenetic chamber angle is the result of abnormal migration or differentiation of the neural crest-derived mesenchymal cells. However, only some patients with goniodysgenetic chamber angles will demonstrate glaucoma, which is referred to as dysgenetic or developmental glaucoma and which occurs earlier in life than open-angle glaucoma. Goniodysgenesis is a fetal maldevelopment or underdevelopment of the iridocorneal angle, resulting in an immature angle.[8] Typically gonioscopic findings are: (1) high location of the iris root at the level of Schwalbe's line, the trabecular meshwork, or the scleral spur[9]; (2) abnormal remnants that cover the angle recess and the trabecular meshwork; (3) abnormalities in the appearance of the trabecular meshwork (for example, in color or width); (4) the absence of the angle recess and prominence or displacement of Schwalbe's line; (5) loops of vessels from the major arterial circle; (6) numerous iris processes; and (7) a narrow or irregular ciliary body band.

Iris hypoplasia or rupture (corectopia), ectopic pupils (polycoria), and tissue strands or adhesions between the iris and the cornea (peripheral anterior synechiae) are AS findings and can be associated with goniodysgenesis, but it is known that goniodysgenesis cannot explain the occurrence of glaucoma per se. Hence, the described goniodysgenesis in MCOR patients with anterior insertion of the iris root alone does not necessarily lead to the development of glaucoma. Patients with MCOR may demonstrate late-onset developmental glaucoma.[10] All reported deletions to date are different from each other, which suggests independent events. The region seems to be prone to nonrecurrent microhomology-mediated deletions. The deletion event reported herein might have been mediated by the Alu repeat elements that overlap with the breakpoint regions.[1] In fact, the proximal and distal regions share a 15-bp long identical nucleotide sequence that is part of some Alu repeat subfamilies. Interestingly, this homologous sequence also is present in proximity of the breakpoints of the smallest deletion reported,[1] and it recurs 11 times (its reverse complement 8 times) in the region where deletions have been described.

In conclusion, the rare chromosome 13q-related microcoria can be associated with a phenotypic spectrum of variable severity. Mild MCOR features require regular, lifelong screening for glaucomatous changes despite the underlying pathogenesis being unknown

to date. Deletion size does not seem to correlate with phenotypic manifestations.

Christina Gerth-Kahlert, MD[1,*]
Jordi Maggi, MSc[2,*]
Marc Töteberg-Harms, MD[1]
Amit Tiwari, PhD[2]
Birgit Budde, PhD[3]
Peter Nürnberg, PhD[3,4,5]
Samuel Koller, PhD[2]
Wolfgang Berger, PhD[2,6,7]

[1]*Department of Ophthalmology, University Hospital Zurich, Zurich, Switzerland;* [2]*Institute of Medical Molecular Genetics, University of Zurich, Schlieren, Switzerland;* [3]*Cologne Center for Genomics, University of Cologne, Cologne, Germany;* [4]*Center for Molecular Medicine Cologne, University of Cologne, Cologne, Germany;* [5]*Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases, University of Cologne, Cologne, Germany;* [6]*Zurich Center for Integrative Human Physiology, University of Zurich, Zurich, Switzerland;* [7]*Neuroscience Center Zurich, University and ETH Zurich, Zurich, Switzerland*
[*]*Both authors contributed equally as first authors.*

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Author Contributions:
Conception and design: Gerth-Kahlert, Maggi, Tiwari, Koller, Berger
Analysis and interpretation: Gerth-Kahlert, Maggi, Töteberg-Harms, Tiwari, Budde, Nürnberg, Koller, Berger
Data collection: Gerth-Kahlert, Maggi, Töteberg-Harms, Tiwari, Budde, Nürnberg, Koller, Berger
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Overall responsibility: Gerth-Kahlert, Maggi, Töteberg-Harms, Koller, Berger

Correspondence:
Christina Gerth-Kahlert, MD, Department of Ophthalmology, University Hospital Zurich, Frauenklinikstrasse 24, CH 8091 Zurich, Switzerland. E-mail: christina.gerth-kahlert@usz.ch.

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